

Aerobic Bacteriology (615) 262-6362

Introduction

The Aerobic Bacteriology Section serves primarily as a referral laboratory for bacteria that are unusual or difficult to identify. In this context, aerobic bacteriology refers to the examination of a wide variety of microorganisms. Reference cultures will be accepted from public and private health care providers. Pure culture isolates are required for serotyping and identification of reference specimens. Clinical specimens are accepted for the isolation and identification of a specific pathogen if this testing is unavailable at the sending facility. Refer to Chart II - 1 AEROBIC SPECIMENS REQUIRING SPECIAL HANDLING.

Refer to sections on *Bordetella* (whooping cough), *Legionella*, Gonorrhea, and *Streptococcus* Group A for specific information on these organisms.

Services available in the Aerobic Section include, but are not limited to, the following:

- Serotyping of *Neisseria meningitidis* from sterile sites.
- Culture and direct fluorescent antibody staining of nasopharyngeal specimens for *Bordetella pertussis*.
- Biochemical identification of non-fermentative gram-negative bacilli and fermentative gram-negative bacilli not included in the family *Enterobacteriaceae*, *Vibrionaceae*, or *Aeromonadaceae*.
- Culture and direct fluorescent antibody staining for *Legionella*.
- Grouping of beta hemolytic streptococci and biochemical identification of clinically significant strains of other gram-positive cocci *on referred isolates only*.
- Biochemical identification or confirmation of any bacterial isolate (other than anaerobes, enterics, or mycobacterium) that is unidentifiable at the local level, due to unusual or special requirements of the organisms. These may include growth requirements, aberrant biochemical results, additional test requirements, as well as the rarity or hazardous nature of the suspected organism.

The Aerobic Bacteriology Section does not perform antimicrobial susceptibility testing for patient treatment.

The Tennessee Medical Laboratory Act requires that laboratory directors send isolates of *Brucella* species, *Corynebacterium diphtheriae*, *Francisella* species, *Francisella tularensis*, **Haemophilus influenzae*, *Legionella* species, *Listeria* species, **Neisseria meningitidis*, **Streptococcus* Group A, **Streptococcus pneumoniae*, and *Yersinia pestis* to the Tennessee Department of Health (TDH) Laboratory for surveillance purposes. Whatever the source, cultures of *L. monocytogenes* from all sites are requested.

*Sterile sites only

All specimens forwarded to the CDC must be accompanied by a clinical history documenting the need for special testing. Services available at the Centers for Disease Control and Prevention (CDC), through the Aerobic Section, include:

- Serotyping of certain bacteria under specific circumstances.
- Toxigenicity testing of *Corynebacterium diphtheriae*.
- Direct fluorescent antibody staining for *Leptospira*.
- Pulsed field gel electrophoresis (PFGE) for coagulase-positive staphylococci from documented outbreaks. A representative sample of the isolates will be pulsed (3-5 isolates.)
- Typing of *Streptococcus pneumoniae* isolates from patients who have received pneumococcal vaccine or from documented outbreaks.
- Antimicrobial susceptibility testing under special circumstances.

Aerobic Bacteriology (Continued)

Chart II - 1 Aerobic Specimens Requiring Special Handling

Organism or Disease	Collection Instructions	Shipping Requirements	Special Requirements
<i>Bacillus anthracis</i> anthrax	Aseptically collect specimens from lesion, contaminated hair products, or sputum. Subculture isolates to blood or nutrient agar slants. Use extreme caution.	Blood culture bottles for blood and spinal fluid, TB plastic tube for sputum. Sterile containers for other specimens.	USE BIOLOGICAL SAFETY HOOD. Do not create aerosols. *Notify Section before shipping.
<i>Bordetella pertussis</i> - whooping cough. Refer to BORDETELLA SECTION.			
¹ <i>Brucella</i> species brucellosis or undulant fever	Aseptically collect multiple blood samples, infected tissues, abscess material, bone marrow, or liver biopsies. Subculture isolates to sheep blood, nutrient or Brucella agar slants. Use extreme caution.	Blood culture bottles, vented and incubated under 5 to 10% CO ₂ . Use sterile container. .	USE BIOLOGICAL SAFETY HOOD. Refrigerated clinical specimen if delay is anticipated. *Notify Section before shipping.
<i>Burkholderia mallei</i> <i>B. pseudomallei</i> melioidosis	Aseptically collect blood, sputum or pus. Subculture isolates to nutrient or infusion agar slant.	Blood culture bottles for blood, TB plastic tube or sputum, sterile container for pus.	*Notify Section before shipping.
¹ <i>Corynebacterium diphtheriae</i> diphtheria	Collect throat or skin lesion swabs. Subculture isolates to Pai or Loeffler's medium.	Sterile container or place on Loeffler's, Pai, cystine blood tellurite, or infusion medium.	Toxigenicity testing Performed at CDC.
¹ <i>Francisella tularensis</i> tularemia or rabbit fever	Collect specimens aseptically. Specimens include material from lesions, lymph nodes, sputum, gastric aspirates, nasopharyngeal washings and blood cultures. Use extreme caution.	Sterile container, no transport medium. Ship frozen on dry ice. Package to prevent leakage.	DO NOT ATTEMPT ISOLATION *Notify Section before shipping.

* Federal regulations require that these organisms must be shipped by a system that requires or provides for notification of receipt, such as registered mail. This is required so that packages can be tracked and undelivered packages may be located quickly. Special safety labeling on the outside of the container is also required.

¹ Tennessee law requires laboratory directors to submit isolates of these organisms to the TDH Laboratory for surveillance purposes.

² Tennessee law requires laboratory directors to submit isolates of these organisms when recovered from a sterile site to the TDH Laboratory for surveillance purposes.

Aerobic Bacteriology (Continued)

Chart II - 1
Aerobic Specimens Requiring Special Handling

Organism or Disease	Collection Instructions	Shipping Requirements	Special Requirements
<i>Haemophilus ducreyi</i> chancroid	Collect specimens from lesions or inguinal bubo and inoculate onto enriched chocolate agar and incubate at 35 to 37°C in 5 to 10% CO ₂ .	Heavy growth of 24 to 48 hour culture scraped with sterile swab, transport as subsurface stabs in chocolate agar.	Primary culture must be done at the local level.
² <i>Haemophilus influenzae</i> - Isolates from sterile sites required for surveillance purposes.			
¹ <i>Legionella</i> species-legionellosis - Refer to LEGIONELLA SECTION.			
<i>Leptospira</i> species leptospirosis	Collect blood during first week of illness, spinal fluid during acute phase, urine (three consecutive specimens) from 2nd week up to several months. Make smears from urine for direct FA and alcohol, acid or formalin fix smears. Hold blood at room temperature.	Obtain media for culture and/or transport from the CDC. Telephone (404) 639-3905 to request. Mail inoculated medium with form PH-1573 to TDH Laboratory for submission to the CDC.	Obtain permission to ship specimen in advance. Complete clinical history required. The TDH Laboratory sends the specimens to the CDC.
² <i>Listeria monocytogenes</i> - Isolates from sterile sites required for surveillance purposes. Isolates from all sites are requested.			
<i>Neisseria gonorrhoeae</i> - gonorrhea - Refer to GONORRHEA AND CHLAMYDIA, DNA PROBE TECHNOLOGY, and GONORRHEA, CULTURE METHOD, SECTIONS.			
² <i>Neisseria meningitidis</i> - Isolates from sterile sites are required for surveillance purposes.			
<i>Staphylococcus aureus</i>	Isolates from documented outbreak. Only coagulase positive staphylococci accepted.	Isolated organisms on nutrient or infusion agar slants.	Documentation must accompany specimens. *Notify Section before shipping. Pulsed Field Gel Electrophoresis (PFGE) is performed at TDH Laboratory Services in Nashville.
² <i>Streptococcus pneumoniae</i> – Isolates from sterile sites are required for surveillance purposes.			
² <i>Streptococcus</i> Group A – Isolates from sterile sites are required for surveillance purposes..			
Miscellaneous Bacteria	Use blood, chocolate or TSA slant or Cary-Blair transport.		

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¹ Tennessee law requires laboratory directors to submit isolates of these organisms to the TDH Laboratory for surveillance purposes.

² Tennessee law requires laboratory directors to submit isolates of these organisms when recovered from a sterile site to the TDH Laboratory for surveillance purposes.

Aerobic Bacteriology (Continued)

Specimen Collection

Aseptically collect specimens from sites such as autopsy material, surgically obtained tissue, urine, and the respiratory and urogenital tract using appropriate techniques for the individual type of specimen. Aseptically collect blood samples and inoculate directly into appropriate commercial blood culture bottles. Preferably, all specimens should be cultured at the local laboratory using recommended isolation procedures. (For exceptions refer to Chart II - 1 AEROBIC SPECIMENS REQUIRING SPECIAL HANDLING.) To ensure purity, isolates should be subcultured onto appropriate media before transportation to the TDH Laboratory.

Isolated organisms should be submitted on non-carbohydrate-containing agar slants such as infusion, nutrient, Trypticase soy, blood, or chocolate. Do not mail broth or plate cultures. A single block of agar from plating media with good growth can be cut out and placed in a sterile tube.

Telephone the Aerobic Bacteriology Section at (615) 262-6362 to make special arrangements in urgent situations or unusual circumstances. **Always** telephone in advance when submitting large numbers of isolates, as in an outbreak situation, or when the organism being submitted is classified as a biologically hazardous organism. For specimens requiring special handling refer to Chart II - 1 AEROBIC SPECIMENS REQUIRING SPECIAL HANDLING.

Specimen Identification

1. Complete all the provider and patient information areas on the Miscellaneous Exam Form PH-1573. Include pertinent clinical information with each specimen.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen, and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form **will not be tested**.

Shipment of Specimens

1. Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. Follow the shipping guidelines of your current carrier or method of shipment.
2. Affix the mailing label (PH-0838), return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.
3. Ship to the Tennessee Department of Health Laboratory in **Nashville**.
4. Use first-class postage on US mail.
5. If *Burkholderia mallei*, *Burkholderia pseudomallei*, *Bacillus anthracis*, *Brucella* species, or *Francisella tularensis* is suspected, telephone the Aerobic Bacteriology Section before shipping.

Aerobic Bacteriology (Continued)

Reporting Procedures and Interpretation of Results

Most cultures are reported within 5 to 10 working days. Mixed cultures or fastidious bacteria may require more time for identification. Final results on isolates submitted to the CDC for confirmation or further testing require a longer interval for completion.

Reporting of Results
Organisms are reported by genus and species.

Organisms are identified to a genus and species level only when culture, morphology, and biochemical test results support the species identification. Genus and species designations are those consistent with designations in the American Society for Microbiology's *Manual of Clinical Microbiology* or according to the *International Code of Nomenclature of Bacteria*. Some organisms encountered in aerobic bacteriology can be identified accurately only to the genus level and are reported as such. Organisms normally encountered as contaminants or those believed to lack clinical significance may be reported only to the genus level especially if the culture was not accompanied by clinical information to the contrary.

Organisms reported as "unidentified" are those which do not fit the description of recognized genera and/or species. These organisms are not routinely forwarded to the CDC for further study unless the nature of the isolate, source of isolation, and/or the clinical history of the patient warrant further identification efforts, or a special request is made to forward the isolate (such a request requires justifying information from the submitter).

The results of all specimen requests are reported to the health care provider who submitted the specimen. In addition, the TDH Communicable and Environmental Disease Services and the health department in the county where the patient lives are sent reports on the following organisms:

Bordetella pertussis
Brucella species
Corynebacterium diphtheriae
Francisella species
Francisella tularensis
Haemophilus influenzae, from sterile body sites
Listeria species
Listeria monocytogenes, from sterile body sites
Legionella species
Neisseria meningitidis, from sterile body sites
Streptococcus pneumoniae, from sterile body sites

The TDH Sexually Transmitted Disease (STD) Control Division, the regional STD representative, and the health department in the county where the patient lives are sent reports on *Haemophilus ducreyi*.

Aerobic Bacteriology (Continued)

Criteria for Unacceptable Specimens

All specimens

1. The specimen was not properly identified with the patient's name/or and the tear strip control number.
2. The patient identifier on the specimen did not exactly match the identifier on the form.
3. The specimen was broken in transit.

Clinical specimens

1. The type of specimen was improper for the test requested.

Reference specimens

1. The specimen was non-viable.
2. A mixed specimen was submitted.

Miscellaneous Exam Form PH-1573 FRONT OF FORM

SOCIAL SECURITY NO.		TENNACARE NO.		MCO		MISCELLANEOUS EXAM <div style="float: right; font-size: 1.5em; font-weight: bold;">A 349473</div>							
MEDICARE NO.		RECORD FOLDER NO.		DATE REPORTED				DATE/TIME RECEIVED					
PATIENT'S NAME - LAST, FIRST, MIDDLE				SPOUSE - FIRST NAME		COLLECTION DATE		DATE OF ONSET					
STREET AND NUMBER								SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER _____ PATHOGEN/DISEASE SUSPECTED/SYMPTOMS					
TOWN		STATE		ZIP		EXAMINATION REQUESTED							
DATE OF BIRTH		RACE		ETHNICITY		SEX		PHONE NO.					
COUNTY NO.		COUNTY NAME				SITE NO.		RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO STATE/COUNTRY					
PLEASE FILL OUT SHADED AREA COMPLETELY TN. DEPT. OF HEALTH DIV. OF LAB SERVICES PRINTED BY STANDARD REGISTER U.S.A. ZIPSET® <div style="display: flex; align-items: center; justify-content: center;"> <div style="writing-mode: vertical-rl; transform: rotate(180deg); font-weight: bold; margin-right: 5px;">REPORT TO</div> <div style="border: 1px solid black; padding: 5px; text-align: center;"> NAME ADDRESS CITY STATE ZIP CODE </div> </div>								EXAMINATION RESULTS <input type="checkbox"/> SUBMITTED TO REFERENCE LABORATORY FOR EXAMINATION. RESULTS FORTHCOMING.					
										EXAMINED BY: _____ RDA-1160			
												TESTING LABORATORY LOCATION CODES J = JACKSON BRANCH LAB, 295 SUMMAR DRIVE, P.O. BOX 849, JACKSON, TN 38302-0849 - DR ORISTYNE WALKER, DIRECTOR K = KNOXVILLE BRANCH LAB, 1522 CHEROKEE TRAIL, P.O. BOX 59019, 37950-9019, KNOXVILLE, TN- DR MICHAEL W. KIMBERLY, DIRECTOR N = NASHVILLE REFERENCE LAB, 630 HART LANE, NASHVILLE, TN 37247-0801 - DR MICHAEL W. KIMBERLY, DIRECTOR	
TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES MICHAEL W. KIMBERLY, DR. P.H., DIRECTOR		<input type="checkbox"/> K <input type="checkbox"/> J <input type="checkbox"/> N LABORATORY PERFORMING EXAMINATION											

BACK OF FORM PH-1573

TIGHTEN CAPS SECURELY
SUBMIT IN DOUBLE MAILING CONTAINER

STOOL CULTURES FOR SALMONELLA, SHIGELLA, CAMPYLOBACTER, AND E. COLI 0157

Place two (2) cotton tipped swabs dipped into feces or other specimens and insert into Amies, Stuarts, or Cary-Blair transport medium. Submit the transport medium refrigerated within two (2) days of collection.

INTESTINAL PARASITES: Place amount of feces equal to volume of formalin in container designed for intestinal parasites (5% Formalin)

CULTURE FOR IDENTIFICATION - Submit pure cultures on non-selective media such as trypticase soy agar slants or enriched slants (Blood or Chocolate) when required.

ANAEROBIC ORGANISMS - Submit in semi-solid media such as thioglycollate, overlaid with sterile vaspar to prevent exposing culture to oxygen.

TESTING LABORATORY LOCATION CODES

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Anaerobic Bacteriology (615) 262-6362

Introduction

Anaerobic bacteria are a frequent cause of serious infections. The Anaerobic Bacteriology Section generally accepts only clinical isolates for identification. In certain cases, clinical material for primary isolation is accepted for cultivation of pathogenic microorganisms. Contact the Anaerobic Bacteriology Section regarding submission of these specimens.

Isolation and identification techniques used include cultural procedures, morphological and biochemical characterization, gas-liquid chromatography, and toxigenicity tests for some *Clostridium* species. Anaerobic isolates are not tested for antimicrobial susceptibilities.

Laboratory Services performs toxigenicity testing and primary isolation for *Clostridium botulinum*. Refer to BOTULISM in Section II. The laboratory also performs primary isolation of *Clostridium perfringens* from suspected food-poisoning cases. Refer to FOODBORNE ILLNESS in Section II.

The Tennessee Medical Laboratory Act requires that laboratory directors submit isolates of *Clostridium tetani* and *Clostridium botulinum* to the Tennessee Department of Health Laboratory for confirmation and surveillance purposes.

Specimen Collection

Since anaerobic organisms make up a major part of the body's indigenous flora, clinical specimens for anaerobic culture must be collected by methods that avoid contamination with normal flora. Aspirates collected with a syringe or tissue specimens are recommended for anaerobic culture.

The TDH Laboratory accepts anaerobic organisms isolated from the following sources:

- Aspirated pus.
- Tissue (biopsy, surgery, autopsy).
- Transtracheal aspirates.
- Direct lung aspirates.
- Body fluids.
- Sulfur granules from suspected cases of actinomycosis.

Anaerobic organisms isolated from the sources listed below are unacceptable for testing. If you submit an isolate from one of these sources, include information that establishes its clinical significance.

- Throat, gingival or nasopharyngeal swabs.
- Skin.
- Voided urine.
- Sputum or gastric contents.
- Superficial wounds.
- Rectal swabs, feces or small bowel contents (except for special testing).
- Vaginal or cervical swabs.

Anaerobic Bacteriology (Continued)

The culture must be maintained in an anaerobic environment. Submit a PURE, actively growing culture in a screw-cap tube of liquid or semi-solid media such as motility media, thioglycollate, or chopped meat broth. Pick a single colony and inoculate a tube of media. At the same time, check the oxygen requirement of the organism by streaking a single colony to an aerobic blood plate. Incubate the confirmed anaerobe for 24 to 48 hours or until visible growth is present. Overlay with 3/4 inch of sterile Vaspar *. Tighten the cap and seal securely with parafilm or waterproof tape to prevent leakage.

* Vaspar: Melt together equal portions (w/w) Vaseline and paraffin. Dispense in 3-ml amounts and autoclave at 121°C for 30 minutes. Store at room temperature and melt for use as needed.

Specimen Identification

1. Complete all the provider and patient information areas on the Miscellaneous Exam Form PH-1573. Include pertinent clinical, biochemical, and epidemiological information with each specimen.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen, and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form will not be tested.

Shipment of Specimens

1. Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. Follow the shipping guidelines of your current carrier or method of shipment.
2. Affix the mailing label (PH-0838), return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.
3. Use first-class postage on US mail.
4. Ship to the Tennessee Department of Health Laboratory in Nashville.

Note: Do not mail specimens on plates. Specimens submitted on plates are acceptable only if they are properly closed in an anaerobic transport bag and delivered by courier to the laboratory.

Reporting Procedures and Interpretation of Results

Most anaerobic cultures are reported within 7 working days, but fastidious, slow-growing, or nutritionally deficient organisms or mixed cultures may require several days longer. Reports from the Centers for Disease Control and Prevention (CDC) on cultures forwarded there can be delayed for several months.

Anaerobic Bacteriology (Continued)

Reporting of results
Organisms are reported by genus and species and subspecies when appropriate.

Organisms are identified to genus, species, and subspecies level when appropriate and only if culture, morphology, biochemical, and gas-liquid chromatographic test results support the identification. Genus, species and subspecies designations are consistent with designations in the Virginia Polytechnic Institute's *Anaerobic Laboratory Manual*, the American Society for Microbiology's *Manual of Clinical Microbiology*, and the *International Code of Nomenclature of Bacteria*. Some anaerobes, particularly members of the genus *Clostridium* and many of the non-sporeforming gram-positive rods, can be identified accurately only to the genus level. Generally, *Lactobacillus* organisms are identified only to the genus level.

The results of all specimen requests are reported to the health care provider who submitted the specimen. In addition, the Tennessee Department of Health Communicable and Environmental Disease Services and the health department in the county where the patient lives are sent reports on the following organisms:

Clostridium botulinum.

Clostridium perfringens, if isolated from a foodborne outbreak.

Clostridium tetani.

Criteria for Unacceptable Specimens

1. The specimen was not properly identified with the patient's name and tear strip control number.
2. The patient identifier on the specimen did not exactly match the identifier on the form.
3. The specimen was broken or leaked in transit.
4. The type of specimen was an improper specimen type for anaerobic culture.
5. The specimen was not submitted under proper anaerobic conditions.
6. The transport media was unsatisfactory for anaerobic transport.
7. The specimen was non-viable.
8. A mixed specimen was submitted.

Anaerobic Bacteriology (Continued)

Miscellaneous Exam Form PH-1573

FRONT

SOCIAL SECURITY NO.		TENN CARE NO.		MCO		MISCELLANEOUS EXAM		A 349473	
MEDICARE NO.		RECORD FOLDER NO.		DATE REPORTED		DATE/TIME RECEIVED		▼ LAB NO. ▼	
PATIENT'S NAME - LAST, FIRST, MIDDLE		SPOUSE - FIRST NAME		COLLECTION DATE		DATE OF ONSET			
STREET AND NUMBER									
TOWN				STATE		ZIP			
DATE OF BIRTH		RACE		ETHNICITY		SEX		PHONE NO.	
COUNTY NO.		COUNTY NAME				SITE NO.			
NAME									
ADDRESS									
CITY		STATE		ZIP CODE					
PH-1573 REV. 11/00		TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES MICHAEL W. KIMBERLY, DR. P.H., DIRECTOR		<input type="checkbox"/> K <input type="checkbox"/> J <input type="checkbox"/> N		LABORATORY PERFORMING EXAMINATION		EXAMINED BY:	
SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER		PATHOGEN/DISEASE SUSPECTED/SYMPTOMS							
EXAMINATION REQUESTED									
RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO		STATE/COUNTRY							
EXAMINATION RESULTS									
<input type="checkbox"/> SUBMITTED TO REFERENCE LABORATORY FOR EXAMINATION. RESULTS FORTHCOMING.									
RD-1160									

TIGHTEN CAPS SECURELY
SUBMIT IN DOUBLE MAILING CONTAINER

STOOL CULTURES FOR SALMONELLA, SHIGELLA, CAMPYLOBACTER, AND E. COLI 0157

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INTESTINAL PARASITES: Place amount of feces equal to volume of formalin in container designed for intestinal parasites (5% Formalin)

CULTURE FOR IDENTIFICATION - Submit pure cultures on non-selective media such as trypticase soy agar slants or enriched slants (Blood or Chocolate) when required.

ANAEROBIC ORGANISMS - Submit in semi-solid media such as thioglycollate, overlaid with sterile vaspar to prevent exposing culture to oxygen.

TESTING LABORATORY LOCATION CODES

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